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Mononchoides composticola n. sp. (Nematoda: Diplogastridae) associated with composting processes: morphological, molecular and autecological characterisation

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Summary – *Mononchoides composticola* n. sp. was isolated from compost and is described based on light and scanning electron microscopy, supplemented with SSU rDNA sequence data. It is characterised by the following features: a denticulate ridge in addition to the dorsal claw-like tooth, a small tooth-like swelling at the stegostom base, *ca* 26 longitudinal ridges on the female body, a uterine sac associated with two dumb-bell-shaped pouches, relatively small spicules (30-38 μm long), a simple gubernaculum shorter than half the spicule length, the genital subventral papillae (v6) consisting of three very small papillae, and a long filiform tail (female: 391-550 μm, 18-26 anal body diam.; male: 304-548 μm, 19-30 anal body diam.). Phylogenetic analyses placed the new species together with *M. striatus*, sister to *Tylopharynx foetida*. Since the use of nematodes as functional indicators often relies on the allocation of nematodes to feeding groups, experiments were performed to elucidate the feeding strategy of the new species. Both its ability to move actively to bacterial food sources and to prey on other compost nematodes were tested. *Mononchoides composticola* n. sp. actively moved towards the compost bacterium *Achromobacter*, a taxis that was temperature dependent, and also preyed on other nematodes. Predation was selective, with a higher predation rate on the relatively small and slow-moving *Rhabditella* sp. than on the considerably larger and more motile *Rhabditis* (*Poikilolaimus*) sp. Adults of *M. composticola* n. sp. have a dual feeding behaviour and can apparently alternate between bacterial and nematode prey.

Keywords – behaviour, compost, description, free-living nematode, molecular, morphology, morphometrics, new species, phylogeny, taxonomy.

Until recently, knowledge of the nematode assemblages and population dynamics associated with composting processes was completely lacking. The nematode community in a Controlled Microbial Composting (CMC) process was analysed in Steel *et al.* (2010). Compared to many soil ecosystems, nematode succession in compost differs mainly by the absence of K-strategists and by the prominence of diplogastrids. At the beginning of the composting process (thermophilic phase), immediately after the heat peak, the nematode population is primarily composed of bacterial-feeding enrichment opportunists (cp-1) (Rhabditidae, Panagrolaimidae, Diplogastridae) followed by bacterial-feeding (cp-2) (Cephalobidae) and fungal-feeding general opportunists (Aphelenchoididae). At the end of the process, at the most mature stage, the fungal-feeding Anguinidae (mainly *Ditylenchus filimus* Anderson, 1983) were dominant. However, before this final stage, during the cooling and maturation stage, an unknown *Mononchoides* n. sp. became dominant ($\geqslant$30% of nematodes). In view of the high abundance of this undescribed *Mononchoides* n. sp., it is reasonable to suggest that it may have a significant role in the

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compost-associated foodweb. *Mononchoides* Rahm, 1928 is a cosmopolitan, quite common, genus and by far the most diverse genus of the family Diplogastridae *sensu lato* (Sudhaus & Fürst von Lieven, 2003) (= taxon Diplogastromorpha in De Ley & Blaxter, 2002). *Mononchoides* inhabits various terrestrial habitats and is often associated with compost, dung, mud, other decaying materials and with different kinds of beetles. Some *Mononchoides* species have been described from freshwater (*e.g.*, sediment of rivers and lakes) and even marine habitats (*e.g.*, sewage) (Sudhaus & Fürst von Lieven, 2003). Within *Mononchoides*, Calaway and Tarjan (1973) listed 18, Andrássy (1984) 17 and Gagarin (1998) 24 species. Sudhaus and Fürst von Lieven (2003), following Gagarin (1998), considered *Glauximena* Allgén, 1947 synonymous with *Mononchoides*, an approach that is also followed in the current paper. In a comprehensive review on diplogastrid nematodes, also taking into account the detailed stoma morphology (see Fürst von Lieven & Sudhaus, 2000), 43 species were listed within the genus *Mononchoides*. Andrássy (2005) did not accept the synonymisation of *Mononchoides* and *Glauximena* based on the presence of a left subventral plate in the buccal cavity of *Mononchoides* species. Andrássy (2005) accepted only 29 valid *Mononchoides* species and nine valid *Glauxinema* species.

Since the review of Sudhaus and Fürst von Lieven (2003), *M. gaugleri* Siddiqi, Bilgrami & Tabassum, 2004 and *M. megaonchus* Mahamood, Ahmed & Shah, 2007 have been described while *M. tokobaevi* Lemzina, 1990 was not listed in Sudhaus and Fürst von Lieven (2003). Within *Glauxinema* (= synonym of *Mononchoides* according to Sudhaus and Fürst von Lieven, 2003), *G. aquaticum* Gagarin & Thanh, 2006 was recently described (Gagarin & Thanh, 2006). Despite their high diversity and numerical importance, the exact feeding habits of *Mononchoides* species, and indeed of most diplogastrids, are barely known. In general they are considered bacterial feeders and/or predators (Yeates *et al.*, 1993). However, Fürst von Lieven and Sudhaus (2000) observed *Mononchoides* sp. feeding on fungal spores and ciliates. It is also very unclear which stages act as predators and which stages can switch to a bacterivorous mode when prey is absent. Knowledge of feeding type is important for our understanding of nematode ecology and for our use and interpretation of nematode-based indices used in community analyses and environmental monitoring (*e.g.*, Index of Trophic Diversity; Heip *et al.*, 1985).

In this paper we describe the new *Mononchoides* species isolated from compost based on morphology (studied by light microscopy (LM) and scanning electron microscopy (SEM)) and on molecular data (SSU rDNA sequences). Aspects of the feeding ecology of this new species are experimentally tested and the ecological implications of the observed feeding habits are considered.

Materials and methods

Collection and culture

Mononchoides composticola n. sp. was extracted from a compost heap at the Institute for Agricultural and Fisheries Research in Merelbeke near Ghent, Belgium (Plant Science Unit, Growth and Development research area), using a modified Baermann funnel method. The heap was composed of three different feedstock materials: 43% fine wood chippings, 43% dry hay and 14% fresh grass. The compost was prepared according to the CMC method, but no microbial starter was added. A composting process is typically subdivided into three different phases based on the temperature profile: the thermophilic phase (45-75°C), the cooling phase (45°C environmental temperature) and the maturation phase ($\approx$ environmental temperature) (approximately 3, 3 and >10 weeks, respectively, in the composting process studied by Steel *et al.*, 2010). *Mononchoides composticola* n. sp. could be detected during the cooling and maturation phase from day 10 until day 165 of the process. A culture with bacteria (*i.e.*, *Achromobacter* sp.) and nematodes (*Rhabditis* (*Poikilolaimus*) sp. and *Rhabditella* sp.) as food sources was established from one female and one male *M. composticola* n. sp.

Cultures were maintained on agar (bacterial agar 2.7 g 400 ml^{-1} (Oxoid, Basingstoke, UK)) and nutrient agar (1.3 g 400 ml^{-1} (Oxoid) plates containing cholesterol (final concentration of 1 μg ml^{-1} (Sigma-Aldrich, St Louis, MO, USA)). The cultures were kept in an incubator at 25°C and generally handled as described by Brenner (1974).

The nutrient portion of the agar also stimulated the development of the natural bacteria present in the compost. An unknown compost bacterial strain was isolated and cultured in LB-medium (2 g yeast extract (Sigma Cell Culture), 4 g tryptone (Oxoid), 2 g NaCl (Acros Organics, Geel, Belgium), 0.4 g (1 M) NaOH and 400 ml distilled water, autoclaved and stored at 4°C). Subsequently, the compost bacteria used for the feeding experiments were identified to genus level at the Laboratory of Microbiology, Faculty of Sciences, Ghent University. The bacteria used for the feeding experiments belonged to *Achro-*

mobacter, a genus known from aquatic habitats and soil and which can also be found in clinical samples (Coenye *et al.*, 2003).

Morphological characterisation

Type material was collected directly from modified Baermann funnel method extractions in a very small drop of water in an embryo dish. Formalin (4% with 1% glycerin) was heated to 70°C and an excess (4-5 ml) was quickly added to the specimens to fix and kill the nematodes instantly (Seinhorst, 1966). The fixed nematodes were processed to anhydrous glycerin following the glycerin-ethanol method (Seinhorst, 1959 as modified by De Grisse, 1969). Measurements and drawings were prepared manually with a camera lucida on an Olympus BX51 DIC Microscope (Olympus Optical, Tokyo, Japan) equipped with an Olympus C5060Wz camera for photographs.

The holotype was also recorded as a video clip mimicking a multifocal observation through a LM microscope following the Video Capture and Editing procedures developed by De Ley and Bert (2002). The resulting virtual specimens are available at http://www.nematology.ugent.be/vce.html

For SEM, specimens preserved in anhydrous glycerin were transferred to a drop of glycerin in an embryo dish. Two drops of water were added every 10 min for 2 h. A subsequent ultrasonic treatment (8 min) of the specimens in a single drop of water removed particles adhering to the body surface. The specimens were dehydrated by passing them through a graded ethanol concentration series of 20, 50, 75, 95, 100% (1 h each), 100% (overnight) and 100% (20 min, next morning). Afterwards, the specimens were critical point-dried with liquid CO_2, mounted on stubs with carbon discs and coated with gold (25 nm) before observation with a JSM-840 EM (JEOL, Tokyo, Japan) at 15 kV.

The terminology describing the parts of the stoma follows De Ley *et al.* (1995) and that of other structural details of the buccal cavity is in accordance with Fürst von Lieven and Sudhaus (2000), which is an essential paper for the correct interpretation of the morphological structures of the diplogastrid stoma region. The male genital papillae formula used was as proposed by Sudhaus and Fürst von Lieven (2003).

Molecular characterisation

For the molecular characterisation one specimen from each of two different cultures was used. One culture was started from *M. composticola* n. sp. specimens isolated on day 31 of the composting process using agar plates (isolate 'culture, day 31') and the other culture was started from *M. composticola* n. sp. specimens isolated on day 84 of the composting process using the modified Baermann funnel method (isolate 'direct extraction, day 84').

DNA extraction, PCR reaction and sequencing the SSU rDNA were done as in Bert *et al.* (2008). The sequences were deposited in GenBank under the accession numbers GU943511 and GU943512. Additional sequences of diplogastrids for phylogenetic analyses were obtained from GenBank. The SSU rDNA sequences were aligned with Clustal W (Thompson *et al.*, 1994) and manually checked. This resulted in an alignment of 1679 characters of which 445 were parsimony-informative. Differences between sequences were counted using the BioEdit sequence alignment options (Hall, 1999). Bayesian phylogenetic inference (BI) was performed with MrBayes v3.1.2 (Ronquist & Huelsenbeck, 2003). A general time-reversible model with rate variation across sites and a proportion of invariable sites (GTR + I + G) was used, as estimated by PAUP*/MrModeltest 2.0b (Nylander, 2004). Two independent, simultaneous analyses were run for 3×10^6 generations and the trees were generated using the last 10^6 generations, well beyond the burn-in value and the point of convergence between the two runs, the latter confirmed by the average standard deviations of split frequencies which approached zero (<0.0012). Other analyses (maximum parsimony, maximum likelihood and LogDet-transformed distance) were done but did not alter the tree topology (except for the support values) and are not further discussed.

Feeding experiments

Preliminary tests were done to identify the best medium and temperature for successful culturing of *M. composticola* n. sp. Two kinds of feeding/foraging experiments were performed, assessing, respectively, the ability of *M. composticola* n. sp. to detect and actively move to a bacterial food source and its potential to feed as a predator on other compost nematodes. These experiments were born out of expectations based on literature information documenting, or at least suggesting, predatory feeding and bacterivory in other diplogastrid nematodes (Yeates *et al.*, 1993; Moens *et al.*, 2004).

To test directional active movement to bacterial food, a Petri dish (8.7 cm diam.) with a 1% agar layer (bacterial agar 4 g 400 ml^{-1} (Oxoid)) was subdivided into seven parallel regions, *i.e.*, an inoculation zone with three positive regions to one side and three negative regions to the other. At the two most extreme, opposite sides (+3 and −3 region, respectively) of the Petri dish, either a 80 μl drop of the bacteria *Achromobacter* in LB medium or a 80 μl drop of distilled water was placed. Ten nematode specimens were washed in distilled water and added to the inoculation zone in the centre of the plate *ca* 1 h after spotting the candidate attractants. To emphasise the distance moved towards or away from the bacterial food source each nematode was scored: nematodes that had moved towards the bacterial attractant were awarded a positive score; the ones moving in the opposite direction were given a negative score, corresponding to the region in which they were recorded (so individual scores ranging from 0 to +3 and from 0 to −3 for migration towards or away from the bacterial spot, respectively). A Leica Mz95 binocular was used for observations. The movement towards or away from a bacterial food source was scored at 1, 2, 4 and 24 h after incubation. Once inside a bacterial spot, most nematodes tended to stay inside it. This experiment was repeated at six different temperatures (15, 20, 30, 40, 45 and 60°C) with ten replicates of each, always with ten *M. composticola* n. sp. per replicate. For the statistical analyses, only the data at 15, 20 and 30°C were used because the nematodes did not survive the entire 24 h incubation at the higher three temperatures. Replicated G-tests for goodness of fit (Sokal & Rohlf, 1995) were used to test the null hypothesis of random movement and hence no preferential migration of *M. composticola* n. sp. towards bacteria. This should result in a 1 : 1 ratio of nematode numbers inside bacterial and control spots. Differences in rate and degree of migration among different temperature treatments were analysed using repeated measures ANOVA followed by post hoc Tukey HSD tests for unequal number of replicates. These analyses were performed on an Attraction Index (Troemel *et al.*, 1997) calculated for each replicate. In brief, this index sums the scores of each nematode on a plate and divides this sum by the number of nematodes recovered. It thus takes into account the position of all nematodes on a plate. The more positive the index, the closer, on average, nematodes on a plate were to the bacterial spot; the more negative the index, the closer, on average, nematodes were to the control spot.

Secondly, the ability of *M. composticola* n. sp. to feed on other compost nematodes was tested. The experiments were executed with two different candidate prey species, *Rhabditis* (*Poikilolaimus*) sp. and *Rhabditella* sp., with ten replicates of each. Petri dishes were filled with a nutrient agar layer covered with a layer of compost bacteria (70 μl bacteria in LB medium spread evenly). The predator treatments consisted of 15 adult *M. composticola* n. sp., 30 adult *R.* (*Poikilolaimus*) sp. or *Rhabditella* sp. and *Achromobacter* compost bacteria, while controls were similarly incubated but contained no *M. composticola* n. sp. Before inoculation on to the agar plates, nematodes were washed in distilled water to remove adhering debris. Prey and predator nematode numbers were counted after 24 and 48 h. Prey consumption was calculated as the difference between prey numbers remaining in the control and in the predator treatment (Moens *et al.*, 2000). Any small juvenile prey nematodes produced during the experimental incubation were not taken into account for our calculations. The edges, walls and lids of the Petri dishes were checked to ensure that no prey had escaped from the agar layers. Natural mortality of both prey and predator was easily detectable since decay times of dead nematodes were longer than the incubation time of the experiments. The occurrence of empty prey cuticles was treated as removed prey. Statistical analysis of differences in the number of prey remaining after 24 and 48 h between the predator and control treatment used Student *t*-tests in Statistica 6.0. Differences in prey removal between the two different prey species after 24 h were also examined using a Student *t*-test.

Results

Mononchoides composticola[*] n. sp.
(Figs 1-3)

Measurements

See Table 1.

Description

Female

Body long, straight to slightly arcuate ventrally after fixation. Cuticle clearly annulated, *ca* 1 μm at mid-

[*] The specific epithet refers to the type habitat of the new species.

body, with conspicuous longitudinal ridges (*ca* 26 at head level). Lip region continuous with body contour, consisting of six fused lips, each with a small papilla. Stoma *s.l.* longer than wide. First stoma part or cheilostom (= stoma *s.s.*) wide, walls heavily cuticularised. Cheilostom subdivided into several (14-18) narrow, rod-like plates (= cheilorhabdions). Bifurcated apex of cheilorhabdions mostly extending beyond labial contour. Second part of stoma consisting of gymnostom and stegostom, both anisotopic with subventral walls longer than dorsal. Relatively small elliptical aperture of amphid located at gymnostom level. According to Fürst von Lieven and Sudhaus (2000), inner wall of gymnostom bearing two dorsolateral denticles, although these could not be explicitly distinguished in this study. Anteriormost part of stegostom bearing a large, claw-like dorsal tooth, a right subventral pyramidal hooked tooth and a left subventral denticulate ridge. Claw-like dorsal tooth with tip pointing dorsally and with prominent duct of dorsal gland. Posterior part of stegostom (= meta- and telostegostom) forming a broad cylindrical tube (on average 4 μm diam. and 12 μm long). Stegostom cylinder on average three times longer than broad. Tooth-like swelling at base of stegostom. Neck region comprising *ca* 11-17% of body. Pharynx divided into muscular procorpus slightly expanding posteriorly into oval muscular metacorpus with valves and a short, non-muscular, glandular isthmus with non-muscular elongated oval basal bulb without valves. Corpus, isthmus and basal bulb in ratio 3 : 1 : 1. Nerve ring encircling isthmus in anterior half. Excretory pore more posterior (7-13 μm posterior to nerve ring) at transition of isthmus to basal bulb. Reproductive system amphidelphic with both branches equally developed, anterior branch on right, posterior branch on left side of intestine. Ovary long with oocytes arranged in one to two indistinct rows in germinal zone. Uterine sac *ca* 20 μm long, connecting both uteri. Vulval opening anterior to mid-body, barely visible as a small circular pore. Vulva lips weakly cuticularised, not protruding. Vagina muscular, with narrow lumen. Pair of dumb-bell-shaped pouches present at level of uterine sac. Phasmids prominent, located posterior to anus. Tail long, filiform.

Male

General morphology similar to female but body slightly smaller, typically J-shaped after fixation. Anterior part with four additional cephalic setae situated on edge of lip region and elliptical amphidial aperture at gymnostom level. Denticulate ridge of different shape to that of female, but precise structure not unequivocal as seen by LM. Testis single, anteriorly ventrally reflexed. Spicules separate slightly arcuate. Gubernaculum with small distal sleeve and inconspicuous proximal appendage. Nine pairs of genital papillae present: three pairs precloacal, six pairs postcloacal. Genital papillae formula: v1, v2, v3d/v4, ad, ph, (v5, v6(3), v7), pd. Papillae with subventral origin marked as 'v1 to v7', numbered from anterior to posterior. Papilla marked as 'v3d' originating laterally next to spicules, 'v6(3)' consisting of three very small papillae grouped close together. Papillae marked as 'ad' (anterior dorsal papilla) and 'pd' (posterior dorsal papilla) originating subdorsally, ad located close to prominent phasmids (ph) and pd situated at attenuating point of tail. Tail mostly shorter than female, filiform.

Type habitat and locality

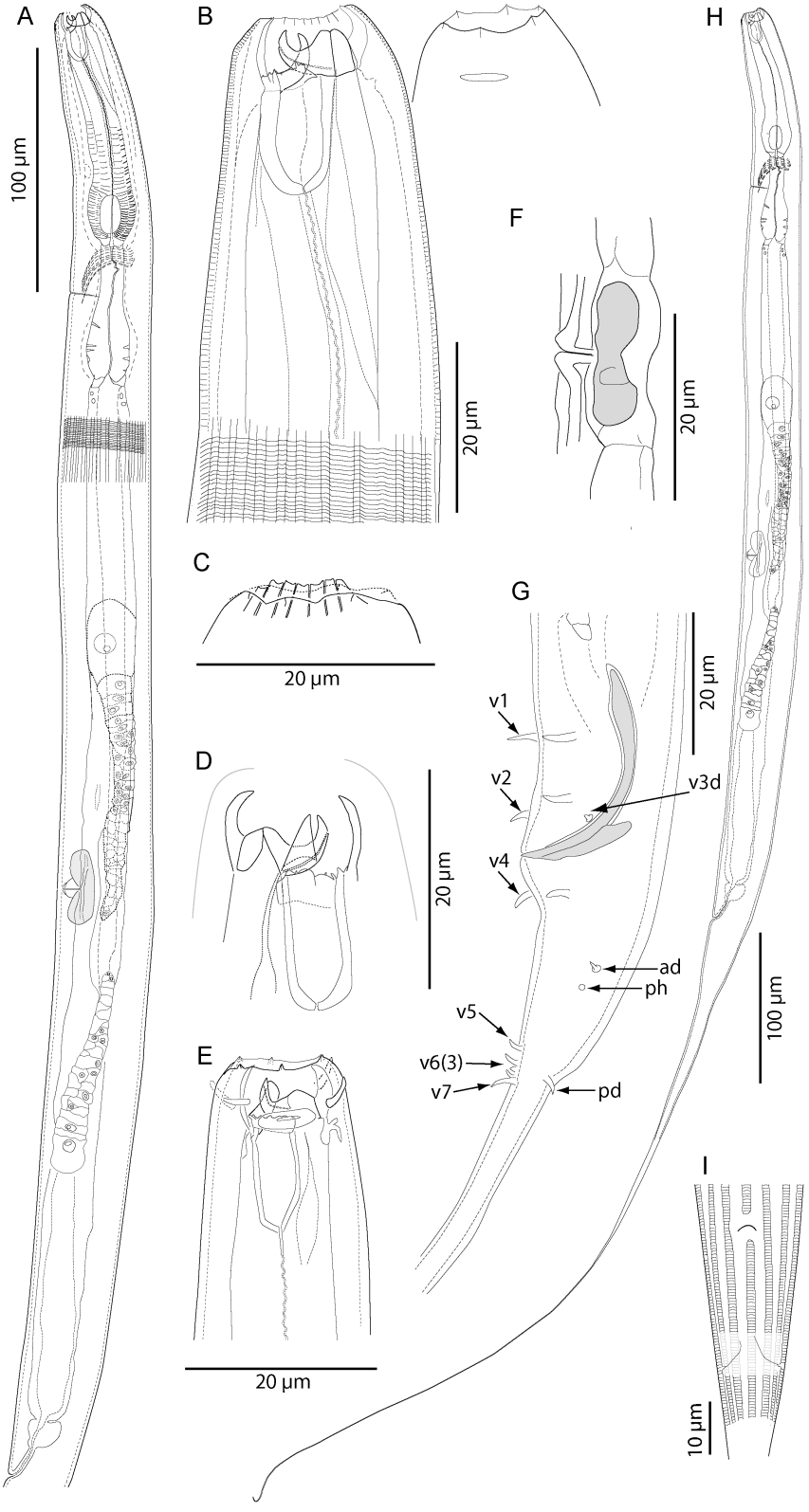
Compost heap at the Institute for Agricultural and Fisheries Research in Merelbeke, Belgium (Plant Science Unit, Growth and Development research area); 50°59′16.62″N, 3°46′30.66″E; altitude: 18.6 m. During the composting process *M. composticola* n. sp. was present from day 10 until at least day 163. The heap was composed of three different feedstock materials: 43% fine wood chippings, 43% dry hay and 14% fresh grass.

Type material

Holotype female, four female paratypes and two male paratypes at the Museum voor Dierkunde (collection number UGMD 104180), Ghent University, Ghent, Belgium. Two female paratypes and two male paratypes at the Wageningen Nematode Collection (WT 3475), University and Research Centre, Landbouwhogeschool, Wageningen, The Netherlands.

Diagnosis and relationships

Mononchoides composticola n. sp. is characterised by a combination of the following morphological features: a denticulate ridge in addition to the dorsal claw-like tooth, a small tooth-like swelling at the stegostom base, *ca* 26 longitudinal ridges on the female body, a uterine sac associated with two dumb-bell-shaped pouches, spicules that are relatively small (30-38 μm), a simple gubernaculum less than half the spicule length long, the genital subventral papillae (v6) consisting of three very small papillae and an especially long filiform tail (female: 391-550 μm, 18-26 anal body diam.; male: 304-548 μm, 19-30 anal body diam.).


A
100 µm
B
20 µm
C
20 µm
D
20 µm
E
20 µm
F
20 µm
G
20 µm
v1
v2
v3d
v4
ad
ph
v5
v6(3)
v7
pd
H
100 µm
I
10 µm

Mononchoides composticola n. sp. is most similar to the *Mononchoides* sp. described by Mahamood *et al.* (2007) but differs by having a longer tail (female: 391-550 *vs* 330-475 μm; male: 304-548 *vs* 204-369 μm), a longer female basal bulb (19-32 *vs* 16-20 μm), a shorter neck in the male (97-113 *vs* 118-143 μm), shorter and smaller spicules and gubernaculum (respectively 30-38 *vs* 37-43 μm and 10-14 *vs* 14-22 μm), the position of the female phasmids (1.4-1.9 *vs* 0.8-1.3 anal body diam. posterior to anus) and the 6th subventral genital papilla comprising three very small subpapillae *vs* a single papilla.

Mononchoides composticola n. sp. is also similar to the following species in having similar morphology and measurements: *M. andrassyi* (Timm, 1961) Gagarin, 1998; *M. flagellicaudatus* Andrássy, 1962; *M. longicaudatus* Khera, 1965; *M. ruffoi* Zullini, 1981 and *M. parastriatus* Paesler, 1946. It differs from *M. andrassyi* by having a denticulate ridge in addition to the dorsal claw-like tooth and the smaller subventral tooth, a shorter female body length (810-1150 *vs* 1008-1420 μm), shorter spicules and gubernaculum (30-38 *vs* 36-42 μm and 10-14 *vs* 17-19 μm, respectively), a longer male tail (18-30 *vs* 12-16 anal body diam.) and by the different shape of the spicules (not cephalated) and shape of the gubernaculum; from *M. flagellicaudatus* by having a denticulate ridge, a larger stoma in the female (14-27 *vs* 13-14 μm), a shorter rectum (0.8-1.5 *vs* 1.8 anal body diam.), a shorter vulva-anus distance (less than two-thirds of tail length), a shorter gubernaculum (10-14 *vs* 15-16 μm), a higher tail/anal body diam. ratio in the male (19-30 *vs* 13-16) and by the lack of the hooked proximal end of the gubernaculum; from *M. longicaudatus* by having a shorter female body (810-1150 *vs* 1100-1400 μm), a shorter female isthmus (13-28 *vs* 48 μm), a shorter male procorpus (34-45 *vs* 62 μm), longer spicules (30-38 *vs* 23-25) without cephalated heads and most obviously by the shape of the dorsal tooth which is not tripartite; from *M. ruffoi* by the lack of an additional serrate cuticularised ring lining the base of the cheilostom and by having a shorter gubernaculum (10-14 *vs* 14-18 μm), more genital papillae (9 *vs* 7 pairs) and a longer male tail (304-548 *vs* 270-310 μm); and from *M. parastriatus* by having shorter and less heavily cuticularised cheilorhabdions, a lower c and b ratio in the male (1.9-2.8 *vs* 2-4 and 4.9-6.7 *vs* 6-8, respectively) and the lack of a pointed distal end to the gubernaculum. *Mononchoides composticola* n. sp. is relatively similar to *M. andersoni* Ebsary, 1986 but differs by having a longer metacorpus (101-115 *vs* 17-34 μm), a shorter stegostom cylinder (10-12 *vs* 18-25 μm), two subventral teeth at the stegostom base and by the presence of males. It also resembles *M. paramonovi* Gagarin, 1998; *M. pulcher* Zullini, 1981 and *M. vulgaris* Gagarin, 2000 morphometrically but lacks the small onchium at the base of the stegostom cylinder. *Mononchoides composticola* n. sp. has a sister relation to *M. striatus* (Bütschli, 1876) Goodey, 1963 based on the limited molecular data but differs by having a lower c ratio in females (1.9-2.8 *vs* 5.2-6.5), shorter spicules and gubernaculum (30-38 *vs* 40-48 μm and 10-14 *vs* 24-26 μm, respectively) and the 6th subventral genital papilla comprising three very small subpapillae *vs* a single papilla.

Phylogenetic analyses

The phylogenetic analysis (Fig. 4) places the two compost isolates of *Mononchoides composticola* n. sp. together with maximal support, sister to *Mononchoides striatus*. The monophyletic *Mononchoides* clade has a maximally supported sister relation with *Tylopharynx foetida* (Bütschli, 1874) Goodey, 1928. The two isolates of *M. composticola* n. sp. only differ in two nucleotides (0.1%) from each other, while they differ in 15 nucleotides (0.9%) from the *M. striatus* sequence obtained from GenBank. The *M. composticola* n. sp. clade and *M. striatus* (however, only represented by one sequence) were both supported by five autapomorphies (Fig. 4).

Fig. 1. Mononchoides composticola *n. sp. from compost. A: Female (holotype) with neck region and reproductive system (without tail); B: Female head region: stoma* s.l. *and first part of procorpus with transverse striations and longitudinal ridges on cuticle plus superficial view of amphidial aperture; C: Rod-like plates (= cheilorhabdions) in cheilostom; D: Detail of female stegostom (lateral view) with dorsal claw-like tooth with prominent duct of dorsal gland, subventral right pyramidal hooked tooth and left subventral denticulate ridge; E: Male head region: stoma* s.l. *and cephalic setae and amphidial aperture; F: Detail of vulva and dumb-bell-shaped pouches at level of uterine sac; G: Detail of male posterior end with separate spicules, gubernaculum and nine pairs of genital papillae (v1, v2, v4, v5 and v7: subventral papillae. v3d: lateral papilla. v6(3): 6th subventral papilla consists of three very small papillae grouped close together. ad: anterior dorsal papilla. pd: posterior dorsal papilla. ph: phasmid); H: Habitus female (holotype); I: Female anus and phasmids (ventral view).*

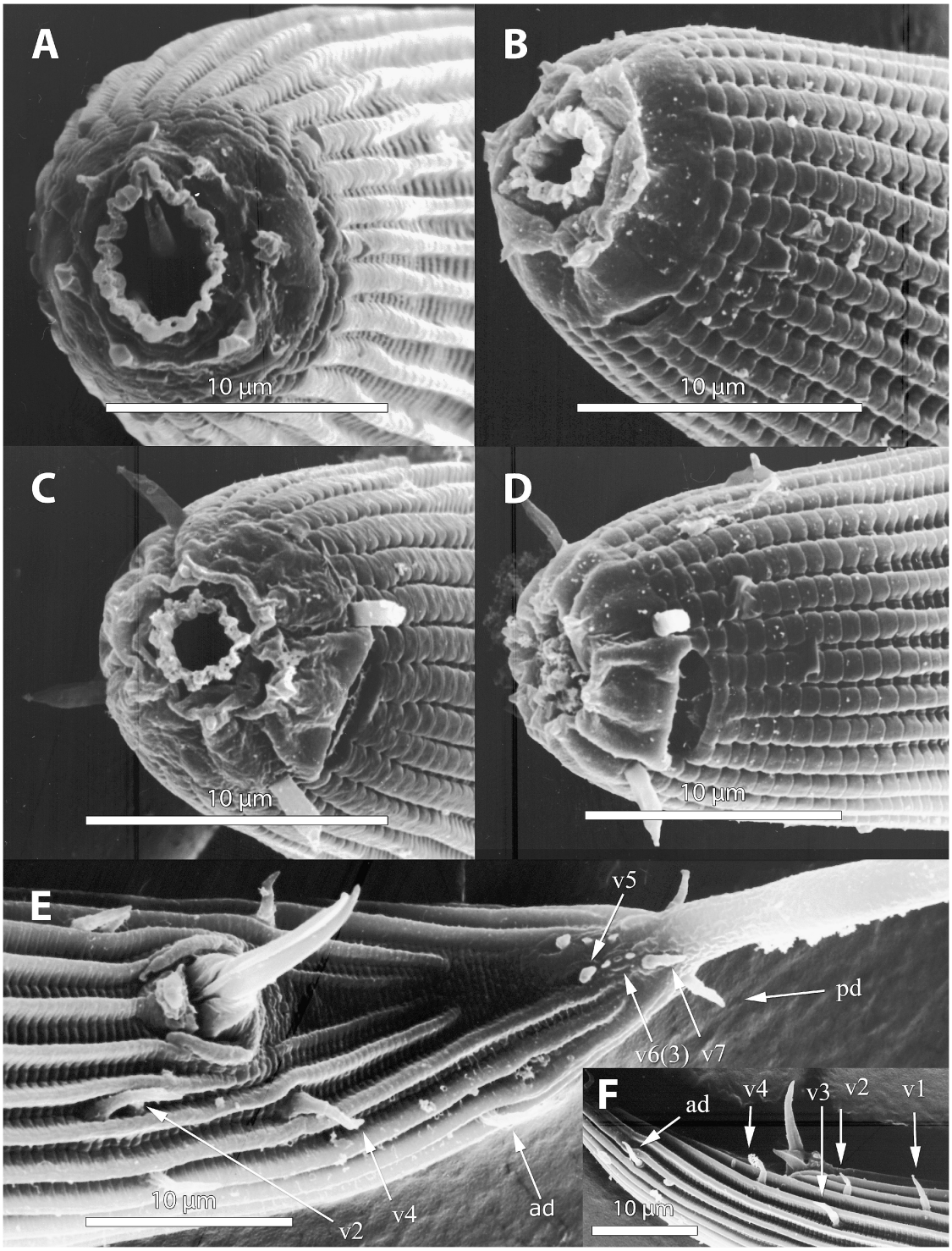


Fig. 2. *SEM photographs of* Mononchoides composticola *n. sp. from compost. A: Female head region showing six fused lips, each with small papilla; B: Elliptical aperture of female amphid; C: Male lip region with four cephalic setae; D: Elliptical aperture of male amphid; E, F: Caudal genital papillae. Abbreviations as in Figure 1.*

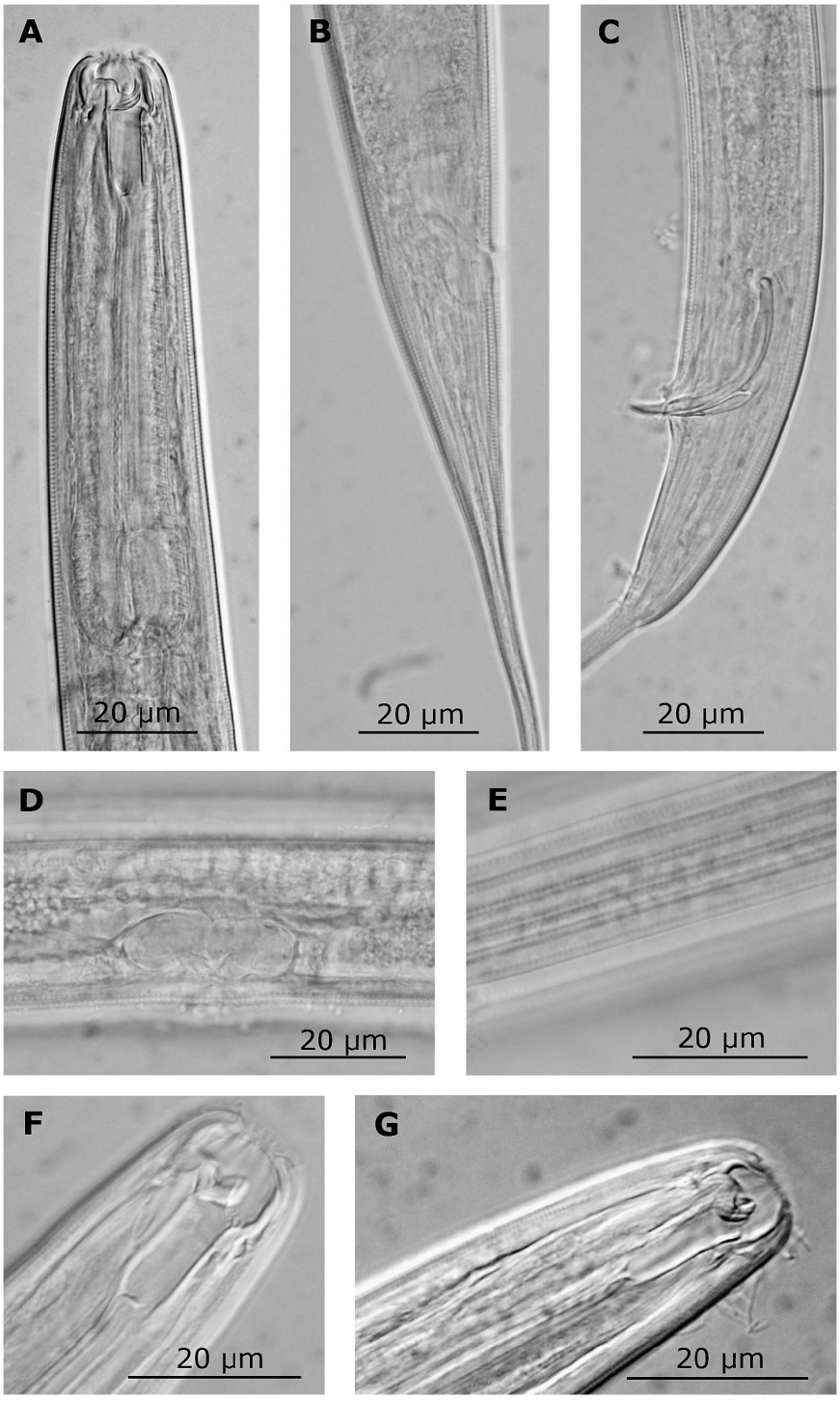


Fig. 3. *LM Photographs of* Mononchoides composticola *n. sp. from compost. A: Female buccal cavity and first part of pharynx; B: Posterior end of female with anus; C: Posterior end of male with spicules and gubernaculum; D: Dumb-bell-shaped pouches at level of uterine sac; E: Annulated cuticle with longitudinal ridges; F: Detail of female stoma with denticulate ridge; G: Detail of female stoma with right pyramidal subventral tooth and claw-like dorsal tooth with prominent duct of dorsal gland.*

Table 1. *Morphometrics of* Mononchoides composticola *n. sp. from compost. All measurements are in μm and in the form: mean ± s.d. (range) CV.*

Character	Female		Male
	Holotype	Paratypes	Paratypes
n	–	30	16
L	1148	1028 ± 105 (810-1150) 10.2	830 ± 88 (761-1029) 10.6
L′	602	555 ± 100 (377-696) 17.9	441 ± 55 (382-572) 12.5
a	31.9	29.8 ± 3.6 (24-36) 12	31.2 ± 4.6 (26.3-40.4) 14.9
b	7.3	7.9 ± 0.9 (5.8-9.4) 12.5	5.6 ± 0.7 (4.9-6.7) 12.7
c	2.1	2.2 ± 0.3 (1.9-2.8) 11.7	2.2 ± 0.3 (1.9-2.8) 13.7
c′	25.4	23.5 ± 2.5 (18.5-26.3) 10.4	21.3 ± 3.8 (18.7-30.5) 17.8
V	33	33.1 ± 3.9 (26.7-40.1) 12	–
V′	63	62 ± 13 (44-86) 20.5	–
Max. body diam.	35.9	34.6 ± 3.6 (30-44) 10.4	25.9 ± 3.2 (16-30) 12.5
Stoma length	22	20 ± 2.4 (14.5-27.3) 12	16.8 ± 2 (13.3-20.9) 12.2
Head diam. (at amphid)	17.4	19 ± 1.5 (16.2-22.6) 8	15 ± 1.6 (13-17.4) 10.8
Stoma length/head diam.	1.3	1 ± 0.1 (0.8-1.3) 9.7	1.1 ± 0.1 (0.9-1.3) 8.8
Amphid width	5.8	6.1 ± 0.9 (5.2-7.5) 14.4	5.3 ± 1.4 (3-8.7) 25.8
Stegostom length	12.2	11.6 ± 1.6 (9.3-16.2) 14.3	10.3 ± 1.3 (8.7-13.9) 13
Stegostom width	5.2	4.0 ± 0.7 (2.9-5.8) 20	3.3 ± 0.6 (3-4.6) 16.8
Cheilostom width	8.7	7.5 ± 1.4 (6-11) 18.2	5.8 ± 1 (5-7) 17.2
Neck length	154	134 ± 9 (117-157) 6.7	116 ± 8.4 (94-128) 7.3
Corpus length	100	90 ± 6 (79-103) 6.2	73 ± 6.8 (52-84) 9.4
Procorpus length	75	67 ± 6 (55-80) 8.4	41 ± 3 (34-45) 7.5
Metacorpus length	25	23 ± 4 (17-34) 17.6	16 ± 3 (14-21) 16.8
Isthmus length	27	20 ± 3 (13-28) 15.7	25 ± 5 (14-32) 17.5
Bulb length	27	24 ± 4 (19-32) 16.3	17 ± 3 (13-23) 16.2
Corpus/isthmus + bulb	1.8	2.2 ± 0.2 (1.7-2.7) 10.4	1.7 ± 0.3 (1.2-2.3) 14.5

Table 1. *(Continued).*

Character	Female		Male
	Holotype	Paratypes	Paratypes
Excretory pore position (EP)	122	109 ± 12* (89-121) 11	94 ± 6 (85-103) 6.2
Nerve ring position (NR)	107	98 ± 11* (83-113) 11.5	77 ± 6 (60-86) 8.3
EP (as % neck length)	77	77 ± 4* (71-81) 5.7	64 ± 4 (60-73) 6
NR (as % neck length)	68	69 ± 2* (66-72) 3.2	52 ± 3 (48-61) 5.8
Tail length	545	473 ± 40 (391-550) 8.5	389 ± 71 (304-548) 18.3
Anal body diam. (ABD)	21.5	20.3 ± 2.2 (17.4-24.9) 10.8	17.3 ± 1.5 (13.9-19.7) 9
Tail/ABD	25.4	23.7 ± 2.4 (18.5-26.4) 10.1	22.5 ± 4.4 (18.7-30.5) 19.3
Anus to phasmid distance	32.5	26.6 ± 5.6* (20-33) 21.2	20.7 ± 3 (17-26) 14.4
Anus to phasmid/ABD	1.5	1.5 ± 0.2* (1.4-1.9) 11.4	1.2 ± 0.2 (0.9-1.5) 17.4
Rectum length	23	22 ± 3 (16-28) 13.2	–
Rectum/ABD	1.1	1.1 ± 0.2 (0.8-1.5) 15.7	–
Tail/rectum	23.5	20.9 ± 7.6 (17.3-30.2) 17.3	–
Gonad length	249	205 ± 46 (132-316) 22.3	–
Posterior gonad	126	103 ± 28 (60-178) 26.9	–
Anterior gonad	123	102 ± 22 (66-145) 21.2	–
Vulval position	382	333 ± 32 (288-392) 9.5	–
Vulval body diam.	37.1	33 ± 4 (25-39) 10.8	–
Vulva to anus distance	262	227 ± 30 (168-307) 13.3	–
Seta length	–	–	4.1 ± 0.7 (2.9-5.8) 18.3
Testis length	–	–	222 ± 30 (175-274) 13.6
Spicule length	–	–	34 ± 1.9 (30-38) 5.6
Spicule/ABD	–	–	2 ± 0.2 (1.6-2.4) 10.2
Gubernaculum length	–	–	12 ± 1 (10.4-13.9) 8.2
Gubernaculum/spicule	–	–	0.4 ± 0.02 (0.3-0.4) 6.4

* $n = 5$.

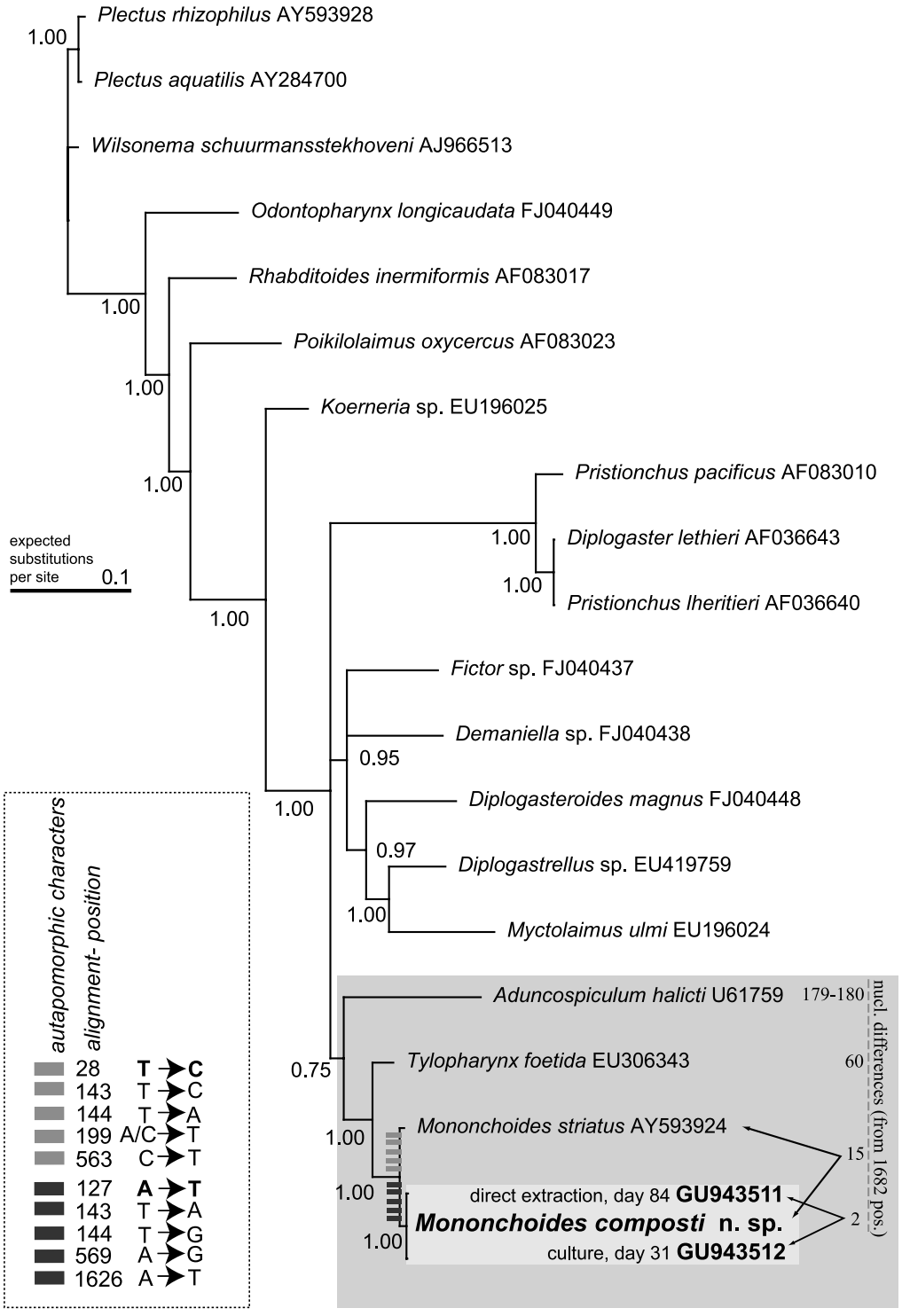


Fig. 4. *Bayesian inference 50% majority rule consensus phylogeny of* Mononchoides composticola *n. sp. and other Diplogastromorpha sequences from GenBank based on SSU rDNA data.* Plectus rhizophilus*,* Plectus aquatilis *and* Wilsonema schuurmansstekhoveni *were designated as outgroup. Branch support values are indicated with Posterior Probability.*

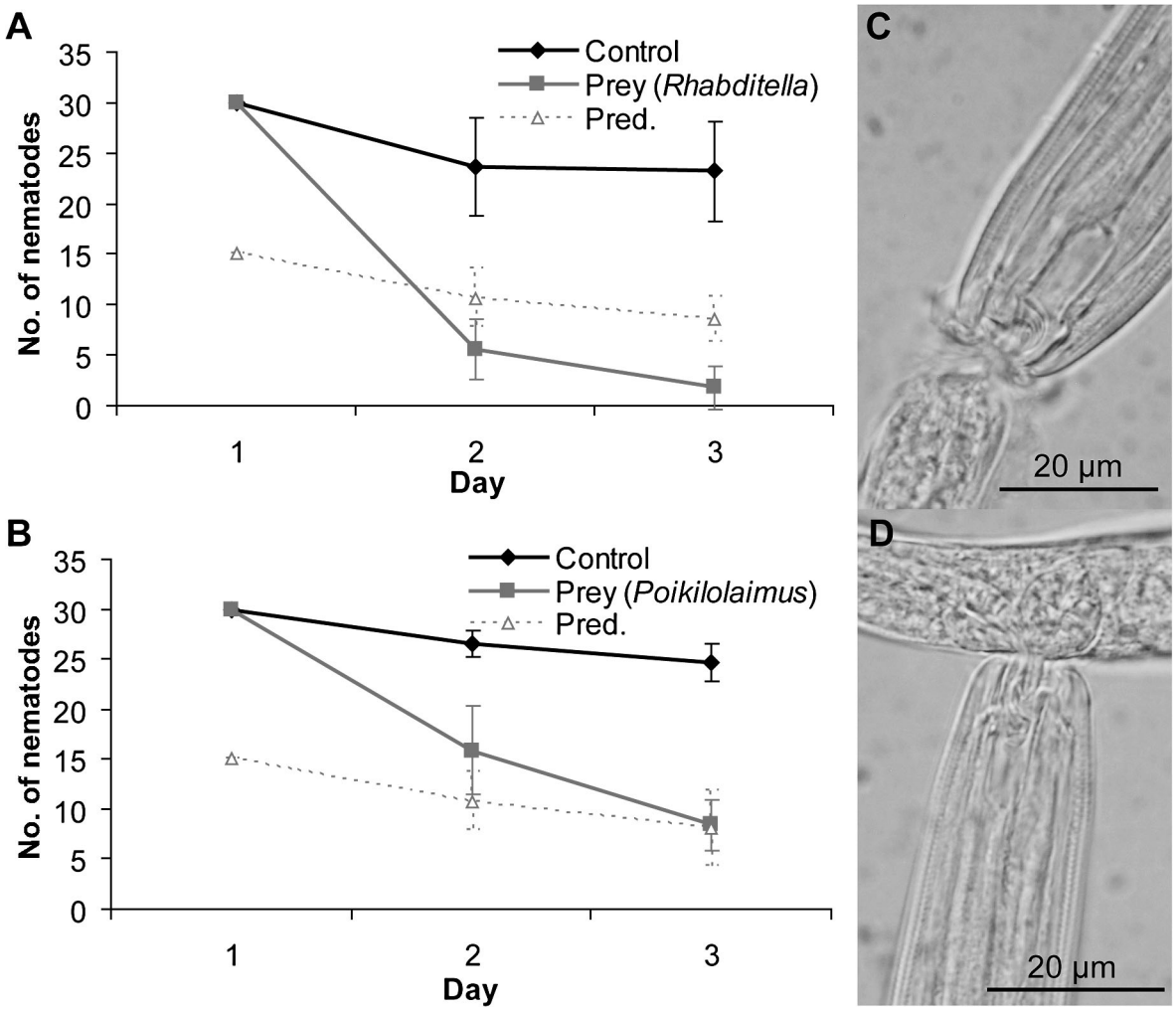


Fig. 5. *Changes in prey and predator numbers over a 2-day incubation with two different prey species. Values are means ± s.d. of ten replicates per treatment. A: Prey =* Rhabditella *sp.; B: Prey =* Rhabditis *(*Poikilolaimus*) sp.; C, D: LM pictures of* Mononchoides composticola *n. sp. feeding on other compost nematodes.*

Feeding experiments

The ratio of *M. composticola* n. sp. reaching the bacterial and control spots after 24 h differed significantly from 1 : 1 at 15, 20 and 30°C ($P < 0.05$), proving the attractiveness of this bacterial strain to the nematode. The positive response to bacteria, when taking into account all nematodes in the plate (Attraction Index), was most pronounced at 20°C and the response showed a significant difference between the temperature treatments of 15 and 30°C ($P = 0.02$). On average 3.86 ± 1.35, 4.8 ± 1.81 and 6.25 ± 2.3 out of ten *M. composticola* n. sp. moved into the bacterial spot after 24 h at 15°C, 20°C and 30°C, respectively, compared to an average 2.71 ± 2.30, 0.4 ± 0.84 and 2.17 ± 1.9 *M. composticola* n. sp. in the control spot.

Figure 5 shows the change in prey numbers over a 2-day period in the presence (predator treatment) and absence (control treatment) of predators. On average, 50 and 80% of prey specimens (*i.e.*, *R.* (*Poikilolaimus*) sp. and *Rhabditella* sp., respectively) were caught in the first 24 h and on average 70 and 92% prey (*i.e.*, *R.* (*Poikilolaimus*) sp. and *Rhabditella* sp., respectively) had been removed after the full 48 h incubation, compared to 18 and 23% of *R.* (*Poikilolaimus*) sp. and of *Rhabditella* sp., respectively, that disappeared in the controls without predators after

48 h. Control and predator treatments showed significant differences ($P < 0.005$) in prey numbers remaining after 24 and after 48 h with both prey species. Over the first 24 h, each *M. composticola* n. sp. consumed on average 1.2 ± 0.3 *R.* (*Poikilolaimus*) sp. and 2 ± 0.7 *Rhabditella* sp. During the next 24 h, *M. composticola* consumed on average 0.9 ± 0.5 *R.* (*Poikilolaimus*) sp. and 1.1 ± 0.3 *Rhabditella* sp. After 24 h, the predation rate of *M. composticola* n. sp. on *Rhabditella* sp. was significantly higher compared to *R.* (*Poikilolaimus*) sp. ($P = 0.02$).

Discussion

Phylogenetic position of *Mononchoides composticola* n. sp. and species characterisation

Our phylogenetic analysis indicates a sister relationship between *Mononchoides* and *Tylopharynx*, confirming the morphologically-based hypothesis that both genera are closely related (Fig. 4). According to Fürst von Lieven and Sudhaus (2000), the right ventro-sublateral tooth was acquired in the ancestral line leading to both genera, whereas the stegostom cylinder and the firmly attached lateral tooth are an apomorphy for *Mononchoides* and *Tylopharynx*. The genus *Tylopharynx* figured prominently in the long lasting controversy over the origins and relationships of Tylenchomorpha (Maggenti, 1963, 1983; Siddiqi, 1980; Poinar, 1983; Andrássy, 1984 *in* Wu *et al.*, 2001), especially because of the apparent similarity of its stoma armature to the tylenchid stylet. Based on the stoma structure of *T. foetida*, De Ley *et al*. (1993) suggested a different origin of the stoma of *Tylopharynx* and the stylet of Tylenchida. The common origin of *Tylopharynx* and *Mononchoides* stoma structures is now well supported, both by morphological and molecular data. The genus *Koerneria* Meyl, 1961 also has a stegostom cylinder, but according to Fürst von Lieven and Sudhaus (2000) it is unclear whether it is homologous with the stegostom cylinder of *Mononchoides* and *Tylopharynx*. Our results indicate that the two structures have evolved independently from each other since *Koerneria* is well separated from *Mononchoides* and *Tylopharynx* in our phylogenetic analysis (Fig. 4).

Within *Mononchoides*, five autapomorphies for *M. composticola* n. sp. as well as *M. striatus* provide evidence of lineage exclusivity for both species. Thus, the species status of *M. composticola* n. sp. is consistent with an amalgamation of evolutionary and phylogenetic species concepts according to Adams (1998). A set of discovery operations to minimise the risk of making systematic errors (Adams, 1998; Nadler, 2002) make this approach both theoretically sound and practically feasible, especially in nematode taxonomy. However, we have to bear in mind that there are currently too few *Mononchoides* sequences available to depict a complete phylogenetic framework for the genus.

Mononchoides is an excellent example of a genus where molecular data are welcome to complete the current morphology-based species descriptions. The morphometric variation in this genus is striking and is clearly influenced by the environment and habitat (*e.g.*, Gagarin, 1998), and in our analyses it turned out that most measurements and ratios have remarkably high coefficients of variation (CV) (mostly between 10 and 20). Evidently, morphometric data alone are insufficient to argue lineage exclusivity in *Mononchoides*. Besides the inherent problem with intraspecific variability, the genus suffers from inadequate descriptions of some species and suggested synonymisations by several authors. Therefore, it is possible that several of the valid names according to Sudhaus and Fürst von Lieven (2003) are actually synonyms of other taxa: for an update of this list with morphometric data, see http://www.nematology.ugent.be/vce.html. Description based on detailed morphological observations (LM as well as SEM), morphometric data from multiple specimens, molecular data and easily accessible digital vouchers would render many dubious species identities more solid.

Feeding behaviour of *M. composticola* n. sp.

Under natural conditions, many diplogastrids are known to feed on bacteria in addition to preying on nematodes (Pillai & Taylor, 1968; Yeates, 1969; Bilgrami & Jairajpuri, 1989; Yeates *et al.*, 1993; Fürst von Lieven & Sudhaus, 2000). Our results are in agreement with this, since *M. composticola* n. sp. detected and migrated to bacterial colonies and preyed upon bacterial-feeding nematodes. Several *Mononchoides* species have been reported as predators on other nematodes: *M. potohikus* (Yeates, 1969), *M. longicaudatus* (Bilgrami & Jairajpuri, 1988), *M. fortidens* (Bilgrami & Jairajpuri, 1988), *M. gaugleri* (Bilgrami *et al.*, 2005), *M. bollingeri* (Goodrich *et al.*, 1968) and *M. changi* (Goodrich *et al.*, 1968). As demonstrated in Figure 5C and D, and as described by Bilgrami and Jairajpuri (1989) and Fürst von Lieven and Sudhaus (2000), *Mononchoides* species pierce the cuticle of their prey by using their movable dorsal tooth, in addition to

pharyngeal suction. They subsequently feed by ingesting some of the body contents of their prey. Our observations suggest that *Mononchoides* species do not always act as an ingester (as described by Yeates *et al.*, 1993) but can also act as a piercer, as described in Bilgrami and Jairajpuri (1989) and Fürst von Lieven and Sudhaus (2000). *Mononchoides* sp. has also been reported feeding on large fungal spores (Fürst von Lieven & Sudhaus, 2000), ciliates (Fürst von Lieven & Sudhaus, 2000), oligochaetes (Small, 1987), tardigrades (Small, 1987), insect larvae (Small, 1987) and amoebae (Small, 1987). Both our experiments illustrate the dual feeding behaviour of adult *M. composticola* n. sp., which can apparently alternate between bacterial and nematode prey. The dual feeding behaviour hampers an unequivocal assignment of *M. composticola* n. sp. to a particular feeding type. Yeates *et al.* (1993) list the genus as bacterial feeder (type 3) and predator (type 5a). In foodweb terminology, an organism feeding at more than one trophic level is an omnivore, yet the term omnivore also represents a feeding type (type 8) in the classification of Yeates *et al.* (1993), where it is not clearly defined. Its use appears to be restricted to certain dorylaimid nematodes. However, assigning a nematode to more than one feeding type (*i.e.*, types 3 and 5a) hinders its inclusion in nematode-based environmental indices such as the enrichment and structure index or the index of trophic diversity.

Difficulties in maintaining *M. composticola* n. sp. in laboratory culture indicate that their high abundance during the composting process (Steel *et al.*, 2010) is intimately linked to aspects of their natural microhabitat in compost, involving a diversity of bacteria and nematodes and a particular temperature regime with temperatures up to 30°C or more for several days. The better we mimicked such a compost environment, the higher the reproduction of *M. composticola* n. sp. (data not shown). Nevertheless, the results of the taxis experiments at different temperatures illustrate that the foraging efficiency of *M. composticola* n. sp. is maintained at temperatures down to 20°C. At still lower temperature (15°C), food (bacteria) finding was significantly reduced. When food (bacteria and prey nematodes) availability was low, *M. composticola* n. sp. often exhibited cannibalism or died while searching for food, as already observed by Pillai and Taylor (1968).

The number of nematode prey decreased significantly after 24 and 48 h for both *R.* (*Poikilolaimus*) and *Rhabditella*. The *per capita* predation rates of *M. composticola* n. sp. on *R.* (*Poikilolaimus*) and *Rhabditella* after 24 h were 1.2 ± 0.3 and 2 ± 0.7, respectively. These rates are low compared to previous studies. Yeates (1969) reported a *per capita* predation rate of approximately 20 prey per 24 h for *M. colobocercus* (Andrássy, 1964) Sudhaus & Fürst von Lieven, 2003 feeding on *Bursilla littoralis* (Yeates, 1969) Andrássy, 1983, *Panagrolaimus australis* Yeates, 1969 and *Acrobeloides syrtisus* Yeates, 1967. *Mononchoides longicaudatus* and *M. fortidens* both consumed on average approximately 10 *Rhabditis* sp. in 24 h (Bilgrami *et al.*, 2005). Predation rates can be influenced by prey density and by interference between different predators in the same habitat (Hamels *et al.*, 2001). Yeates (1969), Bilgrami and Jairajpuri (1989) and Bilgrami *et al.* (2005) demonstrated that *Mononchoides* species show prey density-dependent predation rates.

Predation rates on *Rhabditella* sp. were significantly higher (at least during the first 24 h) than on *R.* (*Poikilolaimus*) sp. This may be related to prey size (on average 650 μm and 800 μm long, respectively) and/or motility. More motile prey have a higher encounter probability with predators (Moens *et al.*, 2000), but may also have a better chance of escape upon a predator-prey encounter (Bilgrami & Jairajpuri, 1989). In our experiment, *Rhabditella* sp. were considerably smaller and less motile than *R.* (*Poikilolaimus*) sp., the latter in fact exceeding *M. composticola* n. sp. in size, suggesting that it was less vulnerable to attack by *M. composticola* n. sp. Prey preference was also reported for *M. longicaudatus*, *M. fortidens* (Bilgrami & Jairajpuri, 1988) and *M. gaugleri* (Bilgrami *et al.*, 2005) and has been attributed to a variety of factors such as prey density, prey secretions, predator ability to wound prey, prey ability to avoid predation, and temperature.

The high abundance of *M. composticola* n. sp. during the composting process, its capacity to prey on other nematodes and its high foraging activity at temperatures below those typical of the compost microenvironment all suggest that the abundance of *M. composticola* n. sp. in maturing compost may be an important factor in determining the biocontrol potential of compost when applied to soils. The microclimate and abundant food sources in compost apparently render compost an ideal 'carrier medium' of *M. composticola* n. sp.

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